The ecological success of Burkholderia terrae BS001 and related strains in the mycosphere

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Dedicated to my grandmother, father (late)
and to my mother.

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Scope of the thesis

Besides containing different abiotic constituents, soil is endowed with a complex community of living organisms whose interactions and functioning provide food for plants and animals. Different functions of global importance, like biogeochemical cycling, mineral weathering, breakdown of recalcitrant materials, nitrogen fixation and solubilization of phosphorus occur in the soil ecosystem. These processes can be exploited in a beneficial way to enhance plant growth and to modulate global ecology, *e.g.* concerning the greenhouse effect. Soil consists of numerous microniches, each of which is occupied by an organism that is best adapted to this environment. It is well established that the soil biota (bacteria, archaea and fungi) plays an important role in the functioning of the system. Among the soil biota, bacteria and fungi are very prominent, as they dominate microbial communities and are key players in the aforementioned processes.

Given the fact that they are so numerous, a range of interactive mechanisms may have evolved between soil bacteria and fungi. Soil is actually one of the most complex ecosystems in which such bacterial-fungal interactions operate. Fungi have particularly important features to make them successful in soil environment, *i.e.* mycelial growth and ability to transport nutrients over longer distances. Both features help the organism to cross air-filled voids and nutrient-poor sites in soil. Also, the thread-like hyphae of fungi are very suitable for penetration into complex soil structures.

Fungi often release carbonaceous compounds into the soil environment, which can be utilized by particular soil bacteria as C sources. These C compounds may exert selective forces that enrich specific microorganisms in their vicinity. This process thus creates novel hospitable niche space for the bacteria that are selected. Besides the provision of nutrients, the influence of the fungus on local bacteria may include pH changes, secretion of inhibitory or stimulatory compounds and adaptation of the soil structure. On the other hand, soil bacteria may also affect soil fungi, such as in the case of the mycorrhization helper effect and detoxification of the fungal milieu. The bacterial-fungal interface in soil is called the mycosphere. Theoretically, the interactions in the mycosphere can either be deleterious, neutral or beneficial to one or both partners. Thus, partners may compete (*e.g.* for nutrients) or antagonize each other. Alternatively, they may take profit of the presence of the partner or cooperate. Although organisms using the different strategies may be found to coexist, those bacteria that developed the “best” mechanisms of interaction may appear as the more successful ones, eventually becoming dominant in the bacterial communities in the mycosphere. At present, our understanding of the processes that take place at the bacterial-fungal interface is limited. Hence, an increased knowledge on the ecological niches (niche space) offered by the fungus and on the bacterial strategies that allow their exploitation is essential to foster our understanding of the interactions. Clearly, the range of possible interactions of bacteria with fungi in nature has not yet been fully investigated. A major hypothesis for soil is the assumption that the heterotrophic soil bacteria, living in a, grossly speaking, carbon-deprived habitat, will interact with soil fungi primarily to obtain carbonaceous compounds. A note of caution here is that the bound carbon in soil is not limiting per every unit site, but that the deprivation of bacteria of carbon sources is mostly due to spatial separation of the bacterial consumers and potential carbonaceous resources.

The current **thesis** examines particular bacterial-fungal interactions in soil, and explores the potential mechanisms/strategies behind the successful ecological interactions that are found. In particular, the interactions between different *Burkholderia* types and the soil saprotrophic fungus *Lyophyllum sp.* strain Karsten are investigated. We thus hypothesized that such bacteria (living in a grossly oligotrophic environment in which carbon starvation is the rule rather than the exception) may have acquired specific capabilities that allow them to quickly interact with emerging fungal hyphae that offer novel ecological opportunities. These ecological opportunities can be summarized as (1) mechanisms of colonization of new sites, (2) improved carbon availabilities and (3) modulated local conditions (pH). Hence, the focus of the work was on the question how bacteria adapt to a soil microhabitat which is created by growing fungal hyphae and what types of mechanisms are involved in these interactions. The thesis will have the following hypotheses and related generic research questions:

Hypotheses

1. Particular groups of soil bacteria have developed specific mechanisms that allow them to benefit from novel ecological opportunities offered by developing soil fungi.
2. Within such soil bacteria, the mechanisms that allow them to be successful may be different. Hence, with respect to the response to soil fungi, parallel paths to enhanced ecological fitness have emerged.

Research questions

1. What are the key mechanisms that allow bacteria to benefit from novel soil niche space that is provided by soil fungi?
2. What different paths have different fungus-responsive soil bacteria taken to enhance their fitness in the microhabitat established by fungi?

The thesis will, accordingly, be split into the following sections in which these general research questions are answered in more detail. The introductory **chapter 1** gives an overview of the current knowledge about bacterial-fungal interactions in soil. The effect of bacteria and fungi on one another and the potential mechanisms known till now are examined and discussed. **Chapter 2** investigates bacterial community establishment in their native and non-native soils in the presence and absence of the soil saprotrophic fungus *Lyophyllum sp.* strain Karsten. Special focus will be on questions like "which organisms from native bacterial communities of different soils would positively be selected by the colonizing fungus *L. sp.* strain Karsten? Are there specific bacterial groups which constitute a major portion of selected bacteria by the fungus irrespective of the origin and fungal-colonized soil?" Fungal-selected bacteria are isolated in this study, in particular those that migrated with the developing hyphae of the fungus in soil. **Chapter 3** then examines the characteristics of particular bacterial strains isolated on *Burkholderia*-selective medium from the fungal migration front. Such so-called community migrators are evaluated for their capacity of single-strain migration through different soils. Along with this ecological feature of migration, all migrators are evaluated in respect of selected genomic, metabolic and phenotypic characteristics. This was done in order to find commonalities which would pinpoint key shared characteristics reporting on local conditions. From among a range of strains, the single-strain migrator *B. terrae* BS001 is selected for further analyses with respect to its potential strategies in the interactions with the fungus. **Chapter 4** then unveils two novel phenotypes (behaviors) of *B. terrae* BS001 in its interaction with *Lyophyllum sp.* strain Karsten. They are (A) inhibition or delay of primordium setting leading to mushroom formation and (B) induction of fungal exudation. The fungal exudates contained glycerol as a major compound which was found to serve as a nutrient and energy source for *B. terrae* BS001. **Chapter 5** summarizes the results regarding the migration of *B. terrae* BS001 along the growing hyphae of different soil fungi. Furthermore, this chapter contains data that point to the protection from some antifungal agents, that *B. terrae* BS001 provides to its fungal partner. This very revealing phenotype exerted by *B. terrae* BS001 might lead to potential applications in biological control of plant pathogens in soil. Given all these interesting features of *B. terrae* BS001, we decided to determine the sequence of its whole genome, as described in **chapter 6**. We found that the draft genome of *B. terrae* BS001 encompasses a staggering 11.5 Mb divided among five replicons. It contained a plethora of genetic systems that are potentially involved in nutrient capture and metabolism as well as interactions with hosts. With the description of the bacterial genome and having an interesting model for bacterial-fungal interactions, a molecular strategy was designed to assess the transcriptional profile of *B. terrae* strain BS001 during its association with *Lyophyllum sp.* strain Karsten. The outcome of this work is summarized in **chapter 7**. The data revealed expression of bacterial genes that are presumably involved in the interaction with the fungal host, *i.e.* biofilm formation and secretion systems. These are well evaluated by this approach to assess the expression in the presence and/or absence of fungus *L. sp.* strain Karsten. **Chapter 8,** finally, summarizes the results obtained about the bacterial-fungal interactions studied here. Some concluding remarks are made along with considerations on the future perspectives in this highly interesting and innovative research area.